American Journal of Hematology 52:319–321 (1996)

Influence of Purified Apoprotein E on Platelet Activation Induced by Serotonin

Olaf Pfennig, Bin Zhao, and Rolf Dierichs
Platelet Research Unit, Institute of Anatomy, University of Münster, Münster, Germany

Serotonin induces platelet activation. Purified apoprotein E of 300 μg/ml prevented morphological alterations of blood platelets stimulated with serotonin (5 μM). Lower concentrated apoprotein E showed no such clear effects. These findings suggest that apoprotein E may liter atherosclerosis by suppressing agonist-induced platelet activation.

Key words: human platelets, apoprotein E, serotonin, aggregometry, electron microscopy

INTRODUCTION

Recent studies indicate that platelets are closely involved in atherosclerogenesis. Serotonin (5-hydroxytryptamine) mediates vascular constriction and induces activation of other platelets, which adhere to atherosclerotic plaques and initiate thrombotic complications [1]. There is evidence that platelet responsiveness to agonists may be modulated by plasma lipoproteins. While native low-density lipoprotein (LDL) and oxidized LDL have a distinct proaggregatory influence on blood platelets [2], reports concerning the effect of high-density lipoprotein (HDL) on platelet aggregability gave conflicting results [3,4]. One of our previous reports demonstrates that whole HDL fails to inhibit serotonin-induced platelet activation [3]. It has been shown that apoprotein E-rich subclasses of HDL (HDL_2), and especially HDL enriched in apoprotein E (HDL-E), inhibit agonist-induced platelet activation [4]. These studies indicate that apoprotein E seems to play a role in platelet activation. The aim of the present work was to study whether delipidized and purified apoprotein E could affect serotonin-induced activation of washed platelets in vitro.

MATERIALS AND METHODS

Purified apoprotein E was a generous gift from the Institute for Arteriosclerosis Research, University of Münster. Briefly, it was extracted from VLDL (d <1.006 g/ml) by delipidization with ethanol ether (2:1). After dissolving in phosphate-buffered solution (PBS), apoprotein E was dialyzed extensively against PBS. The solution was concentrated using an Amicon sample concentrator; protein content was determined according to Lowry [2]. The suspensions of washed platelets were basically prepared as described by Patscheke [5]. Serotonin (5-hydroxytryptamine creatinine sulfate complex) was purchased from Sigma (Deisenhofeng, Germany).

Platelets in suspension were preincubated with apoprotein E (50, 100, 200, 300, 400 μg/ml) at 37°C for 10 min and then stimulated by serotonin (5 μM). In controls, the platelets were treated with serotonin only. During the experiments, all samples were monitored with an APACT aggregometer (Seconic, Tokyo). For ultrastructural study, platelets were prepared as described previously [2]. Ultrathin sections were stained and examined in a Zeiss 109 electron microscope.

RESULTS

Aggregometry revealed that serotonin (5 μM) induced platelet shape change and secretion, which could be suppressed by a preincubation with apoprotein E of 300 μg/ml (Fig. 1). Transmission electron microscopy showed that serotonin led to morphological alterations of platelets (e.g., disc-sphere transformation, granule centralization, incomplete degranulation, and pseudopodia formation). These structural changes indicated an early state of plate-

Received for publication February 20, 1996; accepted March 6, 1996.

Address reprint requests to Dr. Bin Zhao, Institute of Anatomy, University of Münster, D-48149 Münster, Germany.

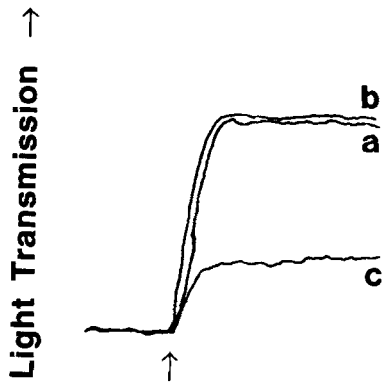

Fig. 1. Aggregometry of platelet suspensions preincubated with apoprotein E (300 μg/ml) with (a) or without (b) following treatment with serotonin. In control (c), the decreased light transmission induced by serotonin indicates disc-sphere transformation of platelets. Arrowhead, switch-on of electronic stirrer.

let activation (Fig. 2A). After preincubation with apoprotein E of 300 μg/ml followed by a stimulation with serotonin (5 μM), few structural changes appeared. Most platelets retained their discoid form, indicating a resting state (Fig. 2B). Lower concentrations of apoprotein E (50–200 μg/ml) failed to prevent the structural alterations induced by serotonin of the same dosage (Fig. 2C). No obvious morphological change was observed after the incubation with apoprotein E of 100–300 μg/ml (Fig. 2D).

DISCUSSION

HDL_2, the apoprotein E-rich subclass of HDL, and especially HDL-E, the apoprotein E-enriched HDL, are reported to inhibit agonist-induced platelet activation [4]. As a major protein constituent of chylomicrons, β-VLDL, and HDL-E, apoprotein E binds to the LDL (B/E)-receptor on cell surface with high affinity [6]. Furthermore other types of binding sites are postulated. Whereas the

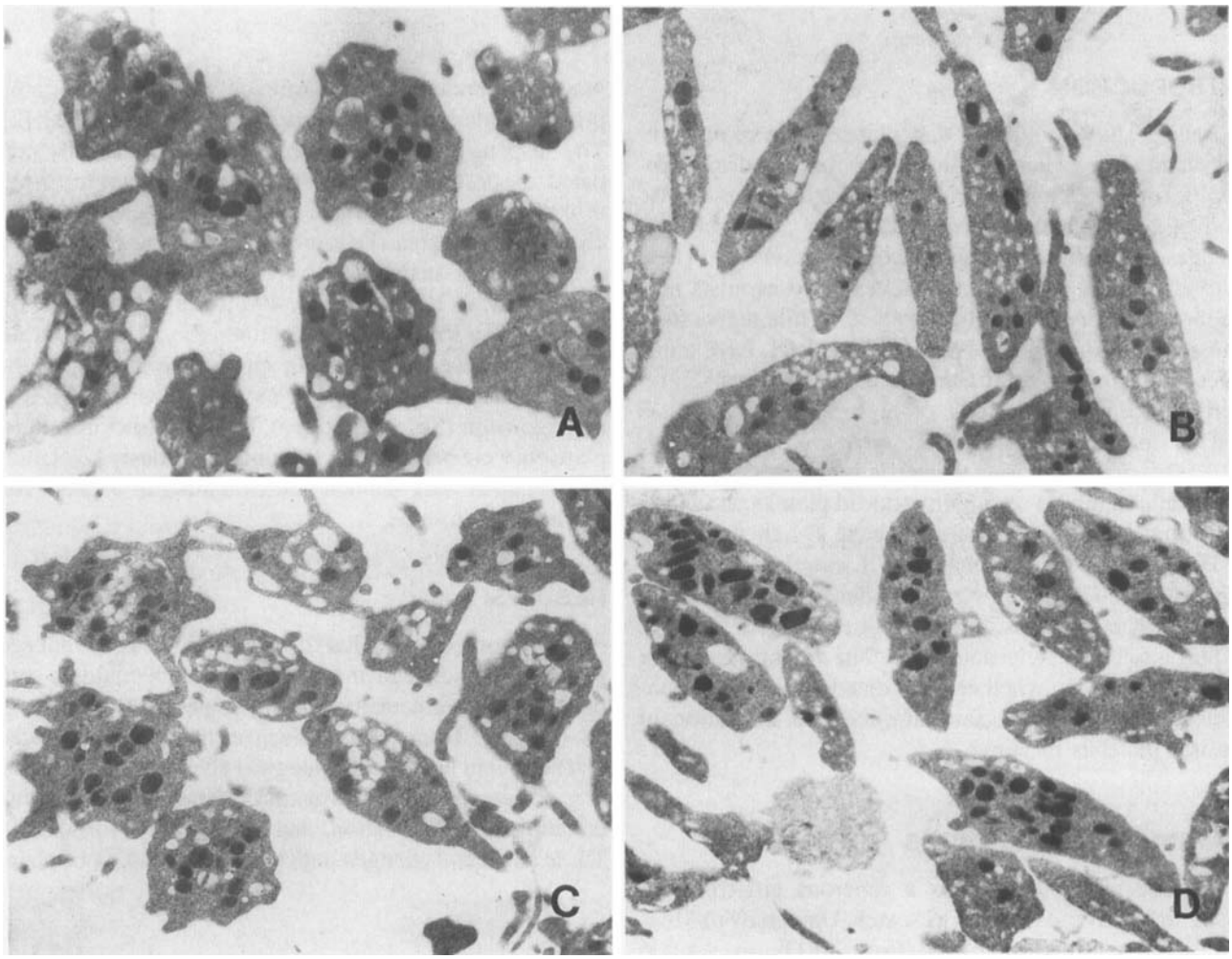

Fig. 2. Transmission electron micrographs of platelets. A: 5 μM serotonin induces shape change, pseudopodia formation, and granule centralization. B: Preincubation with apoprotein E (300 μg/ml) suppresses agonist-induced platelet alteration. In spite of treatment with 5 μM serotonin, most platelets keep their discoid form. C: Apoprotein E (100 μg/ml) does not inhibit shape change, pseudopodia formation, and incomplete degranulation induced by 5 μM serotonin. D: Platelets after incubation with apoprotein E (300 μg/ml).

precise functions of apoprotein E are not sufficiently understood, there is no doubt as to its important role in the metabolism of plasma lipoproteins and in lipid transport [7]. Apoprotein E is suggested to be involved in the reverse cholesterol transport from peripheral tissue to liver by facilitating the acquisition of cholesterol to HDL or by accepting cholesterol from peripheral cells itself [7,8]. Apoprotein E secreted from macrophages supposedly enhances cholesterol efflux from cholesterol-loaded cells [8]. By contrast, apoprotein E-rich VLDL appears to promote the generation of foam cells as a result of an increased endocytosis by macrophages [9]. Moreover, other cell-types involved in atherogenesis, such as lymphocytes and smooth muscle cells, are reported to be influenced by apoprotein E [10].

Our present experiments demonstrated that delipidized and purified apoprotein E could suppress platelet activation induced by serotonin. These in vitro results suggest that apoprotein E plays a relevant role in decreasing platelet aggregability. Some in vivo animal experiments revealed that intravenous administration of purified apoprotein E reduced plasma cholesterol levels [11] and led to a decreased progression of atherosclerotic lesions significantly [12]. These findings correlate with our results and support the hypothesis that apoprotein E is an important factor in the prevention of atherosclerosis. There is evidence that apoprotein E not only enhances the reversed cholesterol transport, but modifies a complex system of cellular function [12]. As the platelet activation process is an important part of this system, we suggest that apoprotein E may be a principal factor when platelet aggregability is suppressed by HDL. The heterogeneous effects of HDL subclasses may be explained that way. Further investigation is necessary to evaluate whether apoprotein E–platelet interaction is a receptor-related event that may contribute to the clinical prevention of atherosclerosis.

ACKNOWLEDGMENTS

We thank Dr. A. Chirazy, Institute for Arteriosclerosis Research, University of Münster, for his kind providing of apoprotein E, and Ms. M. Holling-Rauss, Ms. S. Gleis, and Ms. P. Hassmann for excellent technical assistance.

REFERENCES

1. Fetkovska N: Platelet activation by low-density lipoprotein and serotonin—Effects of calcium antagonists. J Cardiovasc Pharmacol 19:25–28, 1992.
2. Zhao B, Dierichs R, Liu B, Holling-Rauß M: Functional morphological alterations of human blood platelets induced by oxidized low density lipoprotein. Thromb Res 74:293–301, 1994.
3. Pfennig O, Zhao B, Dierichs R: HDL fails to inhibit serotonin-induced platelet activation. Am J Hematol 50:307–309, 1995.
4. Desai K, Bruckdorfer KR, Hutton A, Owen JS: Binding of apoE-rich high density lipoprotein particles by saturable sites on human blood platelets inhibits agonist-induced platelet aggregation. J Lipid Res 30:831–840, 1989.
5. Patscheke H: Shape and functional properties of human platelets washed with acid-citrate. Haemostasis 10:14–18, 1981.
6. Lestavel S, Fruchart JC: Lipoprotein receptors. Cell Mol Biol 40:461–481, 1994.
7. Mahley RW: Apoprotein E: Cholesterol transport protein with expanding role in cell biology. Science 240:622–630, 1988.
8. Basu SK, Goldstein JL, Brown MS: Independent pathways for secretion of cholesterol and apoprotein E by macrophages. Science 219:871–873, 1983.
9. Bates SR, Coughlin BA, Mazzone T, Borensztajn J, Getz GS: Apoprotein E mediates the interaction of β-VLDL with macrophages. J Lipid Res 28:787–797, 1987.
10. Hui DY, Harmony JAK, Innerarity TL, Mahley RW: Immunoregulatory plasma lipoproteins: Role of apoprotein E and apoprotein B. J Biol Chem 255:11775–11781, 1980.
11. Yamada N, Shimano H, Mokuno H, Ishibashi S, Gothoda T, Kawakami M, Watanabe Y, Akanuma Y, Murase T, Takaku F: Increased clearance of plasma cholesterol after injection of apoprotein E into Watanabe heritable hyperlipidemic rabbits. Proc Natl Acad Sci USA 86:665–669, 1989.
12. Yamada N, Inoue I, Kawamura M, Harada K, Watanabe Y, Shimano H, Gotoda T, Shimada M, Kohzaki K, Tsukada T, Shiomi M, Watanabe Y, Yazaki Y: Apoprotein E prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits. J Clin Invest 89:706–711, 1992.